The Uptake of Digestion Products by *Drosera*

By Dr. Graeme Chandler

(Continued from last issue)

Bacteria are associated with all of the carnivorous plant genera, and it was reported that the degradative activity of the bacteria associated with *Darlingtonia* and *Heliamphora* provide the only means available for digesting insects. In other carnivorous genera, there are conflicting reports as to whether entrapped prey are digested by bacteroid enzymes or enzymes secreted by the plants.

Reports of proteolytic enzyme activity associated with *Drosera* are numerous; however, many authors give different pH optima for their particular enzyme. This indicates that possibly the enzymes are of bacterial origin and reflect different bacterial populations associated with *Drosera* at different times. Accordingly, we looked at the bacteria associated with the mucilagenous material on the glandular hairs of *Drosera binata* to see whether or not they produced extra-cellular proteases.

Mucilagenous material was streaked onto agar plates and left to grow for 48 hrs. The five most rapidly growing bacteria were isolated and then examined for proteolytic activity in two ways. One way involves letting bacteria grow in vials in a 10% gelatin solution containing essential salts for 48 hrs. and then placing the vials in the freezer. If the bacteria are producing extracellular proteases, then the gelatin solution will not freeze because of gelatin hydrolysis. The second method is more complicated and more reliable and it involves the bacterium *Salmonella typhimurium*. Certain strains of *S. typhimurium* produce what is termed colicin, which is a diffusible protein toxic to many bacteria as it prevents oxidative phosphorylation, inhibits protein synthesis and/or causes degradation of bacterial DNA. Bacteria which produce extracellular proteolytic enzymes are insensitive to colicin as the toxic factor is hydrolyzed by the proteolytic enzyme. Single colonies of the bacteria isolated from *Drosera* tentacles were plated on agar plates and at the same time *Salmonella* was also plated at varying distances. Growth was continued for 24 hrs. and then the agar flipped over to expose a new sterile surface. An overnight culture of *E. coli* (sensitive to colicin) was surface seeded and growth continued for 24 hours. The plates were then examined for the growth of *E. coli*. If the *E. coli* grows above a *Salmonella* colony, then the other plated bacteria is producing an extracellular protease. All bacteria isolated from *Drosera* tentacles have produced extracellular proteolytic enzymes.

Leaves of *D. binata, D. auriculata* and *D. whittakeri* were examined for proteolytic enzyme activities over broad pH spectrums using three assay methods. The three methods were the ninhydrin technique where the increase in alpha-amino nitrogen is recorded as a complex with the ninhydrin reagent; the second method used casein as a substrate and hence the increase in absorbance at E 280 can be recorded. The third method involved using ^{14}C-labelled protein and we could measure the ^{14}C-peptides or amino acids left in solution after precipitation with T.C.A.

All the plants examined had more than one pH maximum for optimum proteolytic enzyme activity; all species have had a low pH maximum proteolytic activity around pH 3.0 and generally a rather broad band anywhere from pH 4 to pH 8. If proteinaceous material is supplied to the leaves of carnivorous plants, then we tend to get an increase in proteolytic activities right across the board.

The next question was what happens in sterile culture? Seed of *D. binata* was

sterilized and placed into 500 ml agar flasks. When the plants were mature, then they were assayed for proteases and all that we found was one pH maximum indicating the presence of only one proteolytic enzyme in sterile tissue. Using the ^{14}C-labelling assay, the pH maximum was 3.0. This pH maximum is very similar to the proteolytic enzyme reported in both *Nepenthes* and more recently in non-sterile plants of *Drosera capensis*.

Since insects possess a chitinous exoskeleton, we would assume that carnivorous plants would possess a chitinase enzyme to hydrolyze the chitin to the monomeric units of N-acetyl-D- glucosamine. There has only been one report of chitinase activity in carnivorous plants and that was with a non-sterile *Nepenthes* fluid. Leaf extracts of *D. whittakeri* exhibit chitinase activity even though the level of enzyme must be very low. When we examined sterile tissue of *D. binata,* however, we could detect absolutely no chitinase activity at all. So sterile plants have their own proteinase activity but not their own chitinase.

If plants have their own proteinase but no chitinase, this may indicate that bacteria would be necessary if the exoskeleton is degraded. Reports with some carnivorous plants suggest that insect exoskeletons are found in the pitchers of *Nepenthes,* but no long term study has been initiated to see how long the exoskeletons remain. In the growth experiment recently concluded, we tried to determine whether or not spraying the leaves of *Drosera whittakeri* with a bactericide would influence the growth of plants supplied insects. There were four treatments:

1) Plants grown in low nutrient.

2) Plants grown in low nutrient plus bactericide.

3) Plants grown in low nutrient plus insects.

4) Plants grown in low nutrient plus bactericide and insects.

Plants were sprayed every two days with a mixture of penicillin, Streptomycin, Pimafucin and polymyxin, i.e. a bactericide and fungicide. The results were:

	Weight in mg.	*Probability (%)**
Treatment 1	15.9	A
Treatment 2	16.1	A
Treatment 3	18.9	B
Treatment 4	14.9	A

*Values followed by the same letters are not significantly different.

The results suggest that bacteria or rather micro-organisms, since fungi are also associated with leaves of *Drosera,* play a significant role in the nutrition of this plant possibly by providing enzymes necessary for more rapid or complete hydrolysis of insect metabolites.

Carnivorous plants must be able to utilize metabolites bounded by the exoskeleton of prey which are captured; relatively few reports, however, can be found concerning the absorption of nutrients from prey by these plants. Many authors have demonstrated the uptake of radioactive metabolites such as ^{32}Phosphorus and ^{35}Sulphur but not from prey bounded by exoskeleton. Since sulfur of insect origin was shown to be important in the growth experiment with *D. whittakeri,* insects of *Drosphila melanogaster* grown in $^{35}S(SO_4=)$ were supplied sterilized to the plant leaves for 48 hrs. and the labelling pattern of the insect metabolites were monitored. The labelling patterns were reproducible and the sulfur containing insect metabolites identified by extensive chromatography. Sterile insects were supplied to *D. binata* grown in sterile culture. In addition, $^{35}SO_4$ was supplied directly to the leaves of the plants for 48 hrs. Similar experiments were conducted with non-sterilized flies supplied to plants grown under field conditions. The tentacles of these plants are known to harbor a wide variety of micro-organisms.

The results show that the labelling pat-

tern of sterile plants supplied with ^{35}S-labelled insects was qualitatively similar to the labelling pattern to which $^{35}S(SO_4)$ had been administered directly to the tentacle. Also, the labelling pattern resembled in most experiments the same one obtained from the insects alone. Even similar patterns were obtained when non-sterile insects were supplied to plants grown under field conditions.

The similarity of the labelling patterns in all cases does not allow us to determine whether sulphur containing insect metabolites are degraded first to $^{35}S(SO_4)$ or whether the labelled compounds are taken up *per se*. We have attempted to answer this problem by supplying by supplying ^{3}H and ^{35}S-methionine, an amino acid, to the leaves of *D. binata* growing in axenic culture for a given time and then determining the labelling pattern of the plant metabolites. We would expect one of two things to happen:

1) If the methionine is degraded on the leaves, the sulfur atom is then taken up as $SO_4=$ and then we would expect to obtain a labelling pattern similar to that of only $SO_4=$ applied directly to the leaves.

2) On the other hand methionine could be taken up without being degraded and be subsequently metabolized into other compounds. In this case, the % of the ^{3}H-label present in methionine following application of ^{3}H-methionine to the leaves should be the same as the % of the total ^{35}S label following application of equilmolar ^{35}S-methionine.

Accordingly, we supplied both sterile and non-sterile *D. binata* with ^{35}S-sulphate, ^{35}S-methionine and ^{3}H-methionine and determined the plant labelling patterns at ½, 6, 18 and 48 hrs. after the application of the isotopes.

If we consider the plant metabolite patterns at the 6 hr, then we see virtually the same compounds present irrespective of whether ^{35}S or ^{3}H methionine was supplied to the plant. These patterns are substantially different from those obtained when $^{35}S(SO_4)$ was supplied suggesting that methionine is not degraded to SO_4. Further, the % of total ^{3}H and ^{35}S label still present in methionine after 6 hrs. in both sterile and non-sterile plants is similar. We conclude, therefore, that methionine is taken up *per se*.

In summary, we have shown an enhancement of growth of *D. whittakeri* by insects in both nitrogen and sulphur deficient regimes implying that there is uptake of nitrogen and sulfur containing metabolites from the insect to the plant. Application of insects to plants raised in phosphorus deficient regimes did not enhance growth; however, this may be due to the high levels of phosphorus contained in the tubers. Bacteria associated with the leaves of *D. whittakeri* produce both proteases and chitinases and also make significant contribution to the growth of plants grown under a low level of nitrogen. *D. binata* grown in axenic (sterile) culture only produces one protease with pH optimum of about 3.0 and does not exhibit any chitinolytic activity. There is uptake of ^{35}S-insect metabolites under both sterile and field conditions. The uptake by sterile plants demonstrates that mobilization of insect metabolites is independent of bacterial activity, but this process in the field is likely to be insignificant. The uptake of methionine and presumably other compounds occurs *per se*. It is not mediated through degradation to inorganic sulphate.

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CP Field Trip July 1977

by

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In July of 1977, I drove from Orlando, Florida, to San Jose, California. I also drove up the coast of California from L. A. to Oregon, but the area of primary interest here is western Florida, Mississippi and Alabama, where I sought out the CP indiginous to the area.

A short drive from where I was staying outside Orlando, I spotted *Drosera capillaris* in a savannah-like field as well as alongside a small stream with dark muddy banks. The banks were exposed because the stream had obviously receded a few yards. The southern states as well as California were experiencing a drought. The ground of the field in which the plants grew was dried and cracking and all of the *D. capillaris* were small and some sickly looking. I was not hopeful for the prospects of observing other species of CP.

My first day's drive took me to Tallahassee, Florida, where I spent the night. A short drive and ensuing search revealed a water moccasin, some long-legged water birds and a wild clematis plant, but no CP. The following day I planned to reach Mobile, Alabama. Accordingly, I took Rt. 319 South from Tallahassee into Rt. 98 West, passing through towns with names like Crawfordville, Medart, and Sopchoppy. When I stopped along Rt. 98 near Carrabelle, Florida, I found *D. capillaris* growing in dry sandy places as well as wetter areas along ponds and small streams. Also seen was *D. intermedia*. The *D. intermedia* grew only in the wet areas further in from the roadside; the *D. capillaris*, however, extended almost to the road.

I was discouraged because many areas were dried up and I had not yet seen any *Pinguicula* or *Sarracenia*. Further west between Westbay and Destin, Florida, on Rt. 98, I found groupings of *Pinguicula* growing on the sloping intermediary area between the pine forest and the roadside ditch. The soil was very dry and sandy, and the ground was covered with pine needles. The butterworts were of a very pale yellow-green color. Some appeared almost without pigment. A number of plants had very long, thin leaves, and others had more moderately shaped leaves. I guessed that they were *P. lutea*, the "Florida Giant" variety, but there might have been some *P. pumila* among the smaller plants. I was hoping to see *P. primuliflora* and wondered if some of the larger plants might indeed be they.

Growing in the very same kind of habitat slightly further west near Fort Walton Beach, I found *P. planifolia*. This was surprising since I had been looking in whatever wet areas I could find for this plant and had found none. The *P. planifolia* was noticeably different from the first colony of butterworts; they were duller in appearance because of slight reddish pigmentation, especially along the curled edges. Also, the leaves were flatter and wider with only slight in-curling of the edges. The plants were set in the same fairly dry sandy soil — salt and pepper where exposed — with dried grey grass, pine needles, some green clubmoss and short green grasses as ground cover. I was very excited and the plants were quite beautiful — like jewels set in the